a) fragmenting a genomic nucleic acid sample from one or more individuals;

b) physically separating a subset of said nucleic acid fragments based on the size of the fragments;

c) detecting one or more nucleic acid sequence differences, by DNA sequencing, with respect to a reference sequence in the members of said separated molecules of step (b), wherein steps (a)-(c) enrich for and identify a nucleic acid sequence difference with respect to a reference sequence.

159. (Amended) The method of claim 1 wherein said subset of nucleic acid molecules having a sequence that binds to said sequence-specific binding activity comprises fewer than every molecule in the population of nucleic acid molecules in said sample.

REMARKS

Claims 1-3, 57-74 and 145-159 are pending. Claim 2 is cancelled and claims 1, 57, 69 and 159 are amended herein.

Rejections under 35 U.S.C. §112, second paragraph:

Claim 159 is rejected under 35 U.S.C.§112, second paragraph as indefinite. The Office Action states that "it is not clear whether the subset of nucleic acid molecules are less in concentration than every molecule in the sample or they comprise less number of sequence than every molecule in the sample or both." The Office Action states that the metes and bounds of the claim are vague and indefinite. Applicants respectfully disagree.

Applicants submit that because the limitation "less than every molecule in the population" is expressed in terms of molecules in a "population," it is clear that the claim is directed not to concentration, but to the number of molecules in the sample. However, in the interest of avoiding any ambiguity, claim 159 has been amended to recite "wherein said subset of nucleic acid molecules having a sequence that binds to said sequence-specific binding activity comprises *fewer* than every molecule in the population of nucleic acid molecules in said sample." Applicants submit that the amendment adds no new matter. In view of the amendment, Applicants respectfully request the withdrawal of the §112, second paragraph rejection of this claim.

Rejections under 35 U.S.C. §103(a):

Claims 1-3 and 150-153, 157 and 159 are rejected under 35 U.S.C. §103(a) as obvious over Oefner et al. (U.S. Patent No. 5,795,976) in view of Yin et al. (U.S. Patent No. 5,843,633). The Office Action states that Oefner et al. teaches a method comprising the steps of (a) hybridizing a nucleic acid sample with a nucleic acid molecule comprising a sequence-specific binding activity under conditions which permit specific binding, wherein the sample comprises a subset of nucleic acid molecules having a sequence that binds to the sequence specific binding activity, such that the subset of bound nucleic acid molecules is enriched for molecules comprising the sequence recognized by the sequence specific binding activity, and (b) detecting a sequence difference with respect to a reference sequence in the subset of nucleic acid molecules. The Office Action further states that the Oefner et al. reference does not teach a method wherein a subset of nucleic acid molecules having a sequence that binds to the sequence-specific binding activity comprises less than every molecule in the population of nucleic acid molecules in the sample. The Office Action then states that the Yin et al. reference teaches a method wherein a subset of nucleic acid molecules having a sequence that binds to the sequence specific binding activity comprises less than every molecule in the population of nucleic acid molecules in the sample. The Office Action concludes that it would have been obvious to substitute and combine within the comparative hybridization and sequencing method of Oefner et al., the method taught by Yin et al. because Yin et al. states "Sequence similarity is calculated based on a reference sequence, which may be a subset of a larger sequence, such as a conserved motif, coding region, flanking region, etc." Applicants respectfully disagree.

Applicants submit that Oefner et al. detects sequence differences by measuring different HPLC elution times for hybridized nucleic acid molecules with perfect complementarity, relative to hybridized nucleic acid molecules with mismatches. In that method, two nucleic acid samples, a first one of known sequence and the other to be examined for sequence differences relative to the first, are mixed. The mixture is denatured and then allowed to re-anneal. In re-annealing, if there are differences between the known sample and that being examined for differences with the known, duplexes

form that have mismatches, along with duplexes with full complementarity. The difference in HPLC elution of perfectly complementary relative to mismatched hybrids as denaturing stimulus is applied forms the basis of the Oefner et al. method.

Applicants submit that the only teaching of Oefner et al. that could possibly satisfy the claimed requirement for a "sequence specific binding activity" is the complementary nucleic acid strand that forms a duplex, either fully or partially complementary, with each opposite strand molecule in the sample. However, duplexes form between both fully complementary and mismatched sequences. Because it binds both fully complementary and partially mismatched nucleic acid strands, even if the complementary strand is a "sequence-specific binding activity," there is no "subset of bound nucleic acid molecules" as also required by the claims. That is, all nucleic acid molecules in the sample are equal in terms of binding a complementary strand. The binding of every molecule by its full or partial complement does not form a "subset" as the term is defined in the specification. A "subset" as used in the specification and claims requires binding by "less than every molecule in the population" (see specification page 29, line 20 to page 30, line 2 for the definition of a "subset").

Further, because it measures differences in the elution of fully or partially complementary nucleic acids as the strands are *denatured*, the method of Oefner et al. requires that both the known sample and the sample being examined first form *duplexes*. One cannot denature a duplex until one has a duplex to denature. Thus, the method of Oefner et al. only works when all molecules are able to form fully or partially matched duplexes. This does not result in a "subset," because every molecule in the population is bound by a fully or partially complementary strand.

Because Oefner et al is based on the hybridization of all target molecules, whether mismatched or perfectly complementary, Applicants submit that it is simply not possible to "substitute and combine within the method of comparative hybridization and sequencing of Oefner et al." the method of Yin et al. There is no operable way one could "substitute and combine" the teachings of Yin et al. such that the HPLC method of Oefner et al. would have a "subset" of molecules, because the Oefner et al. method **is based on** hybridization between fully or partially complementary molecules – i.e., there is no subset that binds the complementary strand because all sequences bind a fully or

partially complementary strand. Differences in HPLC elution measured by Oefner et al. depend upon the initial hybridization of fully or partially complementary molecules. To somehow introduce the "subset" allegedly taught by Yin et al. will necessarily require a change in the principle of operation of Oefner et al. This is not permitted in constructing a *prima facie* case of obviousness. In re Ratti, 270 F.2d 810, 123 USPQ 349 (CCPA, 1959); MPEP §§2145 and 2143.02.

Further, Applicants submit that the Yin et al. patent relates to an antigen useful for the isolation and characterization of human hematopoietic progenitor and stem cells, and to nucleic acids encoding such antigen. The teaching referred to in the Office Action is that patent's definition of what is meant by "homologous sequences," i.e., nucleic acid sequences homologous to the nucleic acid sequence of the antigen identified in the disclosure. The entire passage cited in the Office Action is reproduced below for convenience:

> Homologous sequences are those with substantial sequence similarity to AC133Ag sequences included within the subject invention, i.e. at least 80%, preferably at least 90%, more preferably at least 95% sequence identity with the nucleotide sequence of the subject DNA sequence. Sequence similarity is calculated based on a reference sequence, which may be a subset of a larger sequence, such as a conserved motif, coding region, flanking region, etc. A reference sequence will usually be at least about 18 nt long, more usually at least about 30 nt long, and may extend to the complete sequence that is being compared. Such homologous nucleic acid sequences will be detected by hybridization under low stringency conditions, for example, at 50°C and 10XSSC (0.9M NaCl/0.09 M sodium citrate) and remain bound when subject to washing at 55°C. with 1XSSC.

Applicants submit that, in this passage, Yin et al. is providing definitional support for claiming nucleic acid homologs of the nucleic acid encoding the novel antigen disclosed in their patent. Yin et al. is simply saying that a nucleic acid molecule that, under the conditions stated, hybridizes with an AC133Ag nucleic acid sequence disclosed in the specification is a "homologous sequence." Not only is there no teaching or suggestion that the so-called "method" taught by Yin et al. can be combined with denaturing HPLC as taught by Oefner et al. in order to enrich for and detect sequence differences, it is not possible to combine them without changing the principle of operation of Oefner et al.

Applicants further submit that it is not clear how the statement in the Yin et al. reference cited for motivation to combine, i.e., that "Sequence similarity is calculated based on a reference sequence, which may be a subset of a larger sequence, such as a conserved motif, coding region, flanking region, etc.," provides the motivation suggested by the Office Action. Taken in context, this statement by Yin et al. simply means that the "reference sequence" referred to in their definition of "homologous sequences" can be part of a longer sequence. The statement recites the words "subset" and "sequence," but it is not at all clear how this statement, taken in *or* out of context, might be construed to provide motivation to modify Oefner et al. as proposed. Applicants request an explanation.

Finally, with regard to the combination of Oefner et al. and Yin et al., Applicants submit that neither reference teaches a method involving contacting a nucleic acid sample with a molecule comprising a sequence-specific binding activity selected from the group consisting of: transcription factors or DNA binding domains thereof; proteins with zinc-finger DNA binding domains; restriction endonuclease DNA recognition domains; sequence-specific antibodies; oligonucleotides complementary to an adapter ligated to a population of DNA molecules; aptamers; peptide nucleic acid molecules; peptides; and affinity resins which recognize DNA having a particular G+C content or methylation status, as required by claim 1 as amended. That is, Oefner et al. teaches, if anything, a nucleic acid as the "sequence-specific binding activity." Yin et al. does not teach or suggest any of the sequence-specific binding activities recited in the claim as amended. The language of the amendment is supported in the specification, and in claim 2 of the claims as filed. The amendment adds no new matter. Because the combination of Oefner et al. and Yin et al. does not teach or suggest all elements of the invention of claim 1, Applicants submit that claim 1 and its dependents (specifically, claims 2-3, 150-153 and 159) cannot be obvious over the suggested combination.

Applicants note that the rejection of claims over the combination of Oefner et al. and Yin et al. included claim 157, which does not depend from amended claim 1. However, independent claim 157 is drawn to a method comprising contacting a nucleic acid sample with a DNA binding domain of a restriction endonuclease that cleaves the nucleic acid sample 300,000 times for fewer, wherein the sample comprises a subset of

nucleic acid molecules having a sequence that is bound by the DNA binding domain, and wherein a bound subset of nucleic acid molecules is retained by the DNA binding domain, such that the subset of bound nucleic acid molecules is enriched for molecules comprising the sequence recognized by the DNA binding domain. Applicants submit that neither Oefner et al. nor Yin et al. teaches or suggests the use, within a method of enriching for and identifying a nucleic acid sequence difference with respect to a reference sequence, of a DNA binding domain of a restriction endonuclease that cleaves the nucleic acid sample 300,000 times for fewer. Because the combination of Oefner et al. and Yin et al. does not teach or suggest all elements of the invention of claim 157, Applicants submit that claim 157 cannot be obvious over the suggested combination.

In view of the above, Applicants respectfully request the withdrawal of the rejection of claims 1-3, 150-153, 157 and 159 over Oefner et al. in view of Yin et al.

Claims 1-3, 57-68 and 145-153, 157 and 159 are rejected under 37 C.F.R. §103(a) as being obvious over Oefner et al. in view of Yin et al. in further view of Gaitanaris (U.S. Patent No. 6,228,939, filed 12/31/97). The Office Action states that Oefner et al. in view of Yin et al. does not teach the method wherein a nucleic acid fragment is operatively linked to a vector and replicating the operatively linked subset to form an enriched collection of replicated molecules. The Office Action further states that the Gaitanaris reference teaches a method wherein a nucleic acid fragment is operatively linked to a vector and replicating the operatively linked subset to form an enriched collection of replicated molecules. The Office Action concludes that it would have been obvious to substitute and combine, within the method of Oefner et al. in view of Yin et al., the method described by Gaitanaris et al. As motivation for the suggested combination, the Office Action cites the statement in Gaitanaris et al. "The invention features a method for identifying a mutagenized mammalian gene." Applicants respectfully disagree.

First, as discussed above, Applicants submit that neither Oefner et al., Yin et al., nor Gaitanaris et al. teaches a method involving contacting a nucleic acid sample with a molecule comprising a sequence-specific binding activity selected from the group consisting of: transcription factors or DNA binding domains thereof; proteins with zinc-

finger DNA binding domains; restriction endonuclease DNA recognition domains; sequence-specific antibodies; oligonucleotides complementary to an adapter ligated to a population of DNA molecules; aptamers; peptide nucleic acid molecules; peptides; and affinity resins which recognize DNA having a particular G+C content or methylation status, as required by claim 1 as amended. Thus, the proposed combination of references cannot render obvious the invention of claim 1 or claims 2-3, 145-153 and 159 that depend from it. Applicants also note that claim 1 and the claims dependent from it do not recite the limitation that the Gaitanaris patent is said to provide.

Second, with regard to claims 57-68, Applicants submit that the cited combination of Oefner et al., Yin et al. and Gaitanaris et al. does not teach a method involving detecting one or more nucleotide sequence differences in the members of the collection by DNA sequencing as required by claim 57 as amended. The amendment is supported in the specification at, for example, page 13, lines 7 and 8. Applicants submit that Oefner et al. teaches detection of sequence differences on the basis of differing HPLC elution times for perfectly versus imperfectly matched nucleic acid strands as a denaturing stimulus is applied. This detection of sequence differences is not DNA sequencing. Further, Applicants submit that neither Yin et al. nor Gaitanaris et al. teach or suggest the substitution of DNA sequencing in a method of enriching for and identifying nucleotide sequence differences with respect to a reference sequence. Applicants therefore submit that the suggested combination of references cannot render obvious the invention of amended claim 57 or of claims 58-68 that depend from it.

With regard to claim 157, also included in the rejection over Oefner et al. in view of Yin et al and Gaitanaris et al., Applicants submit that neither Oefner et al., Yin et al., nor Gaitanaris et al. teaches a method involving contacting a nucleic acid sample with a DNA binding domain of a restriction endonuclease that cleaves the nucleic acid sample 300,000 times for fewer. As such, no combination of the references can render the claimed invention obvious.

In view of the above, Applicants respectfully request the withdrawal of the §103(a) rejection of claims 1-3, 57-68, 145-153, 157 and 159 over the cited combination of Oefner et al., Yin et al. and Gaitanaris et al.

Claims 1-3, 69-74 and 150-159 are rejected under 37 C.F.R. §103(a) as obvious over Oefner et al. in view of Yin et al. in further view of Cabib et al. (U.S. Patent No. 5,912,165). The Office Action states that Oefner et al. in view of Yin et al. do not teach fragmenting a nucleic acid sample by endonuclease digestion or cleavage with an infrequently cleaving restriction endonuclease selected from NotI. The Office Action states that the Cabib et al. reference teaches these elements. As motivation to combine, the Office Action cites the passage in the Cabib et al. reference stating "A complete digestion by a rare cutter endonuclease (e.g., NotI) is used. The latter is presently preferred, since a complete digestion can be repeated to yield identical results in independent trials, whereas partial digestion is random in nature." Applicants respectfully disagree with the Office Action's conclusion of obviousness.

As discussed above, neither Oefner et al. nor Yin et al. teaches a method involving contacting a nucleic acid sample with a molecule comprising a sequence-specific binding activity wherein the sample comprises a subset of nucleic acid molecules having a sequence that binds the sequence-specific binding activity, as required by claim 1. Further, neither Oefner et al. nor Yin et al teaches a method involving contacting a nucleic acid sample with a molecule comprising a sequence-specific binding activity selected from the group consisting of: transcription factors or DNA binding domains thereof; proteins with zinc-finger DNA binding domains; restriction endonuclease DNA recognition domains; sequence-specific antibodies; oligonucleotides complementary to an adapter ligated to a population of DNA molecules; aptamers; peptide nucleic acid molecules; peptides; and affinity resins which recognize DNA having a particular G+C content or methylation status, as required by claim 1 as amended. Applicants submit that Cabib et al. similarly does not provide such a teaching. Thus, the proposed combination of references cannot render obvious the invention of amended claim 1 or claims 2-3, 150-156 and 159 that depend from it.

With respect to this rejection as it applies to independent claim 69 and its dependents, Applicants submit that neither Oefner et al., Yin et al., nor Cabib et al. teaches "detecting one or more nucleic acid sequence differences, by DNA sequencing, with respect to a reference sequence" in the members of the subset of nucleic acid fragments physically separated on the basis of fragment size, as required by claim 69 as

amended. The amendment is supported in the specification at, for example, page 14, lines 20 and 21. Specifically, none of the references teach or suggest the use of DNA sequencing to detect a sequence difference. Oefner et al. teaches the use of denaturing HPLC to detect sequence differences, and neither Yin et al., nor Cabib et al. teach or suggest the use of DNA sequencing in place of denaturing HPLC. Even if they did, which they do not, such substitution would necessarily change the principle of operation of the method of Oefner et al., which is not permitted in constructing a prima facie case of obviousness. Applicants submit that claim 69 and it s dependents 70-74 therefore cannot be considered obvious over any combination of Oefner et al., Yin et al. and Cabib et al.

With respect to claim 157 and it dependent claim 158, Applicants submit that neither Oefner et al., Yin et al., nor Cabib et al. teaches a method comprising contacting a nucleic acid sample with a DNA binding domain of a restriction endonuclease that cleaves the nucleic acid sample 300,000 times for fewer, wherein the sample comprises a subset of nucleic acid molecules having a sequence that is bound by the DNA binding domain, and wherein a bound subset of nucleic acid molecules is retained by the DNA binding domain. Specifically, none of these references, including Cabib et al., teaches the use of a DNA binding domain of a restriction endonuclease that cleaves the nucleic acid sample 300,000 times for fewer. Rather, if anything, they describe the use of a restriction endonuclease, not a DNA binding domain of such a molecule. While a restriction endonuclease can comprise a sequence specific DNA binding domain, a complete restriction endonuclease will not retain a bound subset of nucleic acid molecules, as required by claim 157. Rather, a compete restriction endonuclease will transiently bind, cleave, and release the cleaved fragments of a nucleic acid comprising a recognition site. As such, Cabib et al. does not satisfy the limitations of claim 157 or of dependent claim 158. Applicants therefore submit that no combination of Oefner et al., Yin et al. and Cabib et al. can render obvious the invention of claims 157 or 158.

In view of the above, Applicants submit that claims 1-3, 69-74 and 150-159 are not obvious over the cited combination of Oefner et al., Yin et al. and Cabib et al.

Applicants respectfully request the withdrawal of the §103(a) rejection of these claims over this combination of references.

In view of the above, Applicants submit that all issues raised in the Office Action are addressed herein. Applicants respectfully request the reconsideration of the claims.

Respectfully submitted,

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Date

Kathleen M. Williams, Ph.D.
Registration No. 34,380
Attorney for Applicants
PALMER & DODGE LLP
111 Huntington Avenue
Boston, MA 02199-7613
Telephone: (617) 239-0451
Telecopier: (617) 227-4420

Version of amendments marked to show changes:

1. (Twice amended) A method of enriching for and identifying a nucleic acid sequence difference with respect to a reference sequence comprising:

a) contacting a nucleic acid sample with a molecule comprising a sequence-specific binding activity selected from the group consisting of: transcription factors or DNA binding domains thereof; proteins with zinc-finger DNA binding domains; restriction endonuclease DNA recognition domains; sequence-specific antibodies; oligonucleotides complementary to an adapter ligated to a population of DNA molecules; aptamers; peptide nucleic acid molecules; peptides; and affinity resins which recognize DNA having a particular G+C content or methylation status,

under conditions which permit specific binding, wherein said sample comprises a subset of nucleic acid molecules having a sequence that binds to said sequence-specific binding activity, and wherein a bound subset of nucleic acid molecules is retained by the sequence-specific binding activity, such that the subset of bound nucleic acid molecules is enriched for molecules comprising the sequence recognized by the sequence specific binding activity; and

b) detecting a sequence difference with respect to a reference sequence in the subset of nucleic acid molecules,

wherein steps (a) and (b) enrich for and identify a nucleic acid sequence difference with respect to a reference sequence.

57. (Three times amended) A method of enriching for and identifying nucleic acid sequence differences with respect to a reference sequence comprising:

a) fragmenting a nucleic acid sample from one or more individuals;

b) physically separating a subset of said nucleic acid fragments based on the size of the fragments;

c) operatively linking a fragment created in step (a) or said subset of step (b) with an oligonucleotide or a vector;

d) replicating said operatively linked subset to form an enriched collection of replicated molecules; and

e) detecting one or more nucleotide sequence differences in the members of said collection of step (d) [with a method that detects one or more nucleotide differences with respect to a reference sequence] by DNA sequencing,

wherein said steps (b) and (c) follow step (a) but can occur in either order, followed thereafter by steps (d) and (e) in that order, wherein said steps (a)-(e) enrich for and identify a nucleic acid sequence difference with respect to a reference sequence.

69. (Three times amended) A method of enriching for and identifying nucleic acid sequence differences with respect to a reference sequence comprising:

a) fragmenting a genomic nucleic acid sample from one or more individuals;

b) physically separating a subset of said nucleic acid fragments based on the size of the fragments;

c) detecting one or more nucleic acid sequence differences, by DNA sequencing, with respect to a reference sequence in the members of said separated molecules of step (b), wherein steps (a)-(c) enrich for and identify a nucleic acid sequence difference with respect to a reference sequence.

159. (Amended) The method of claim 1 wherein said subset of nucleic acid molecules having a sequence that binds to said sequence-specific binding activity comprises fewer [less] than every molecule in the population of nucleic acid molecules in said sample.